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## Research Article

# Determination of Late Embryonic Death Rates by Pregnancy Associated Glycoproteins and Rectal Examination in Cows

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**ABSTRACT**

In this study, it was aimed to make the pregnancy diagnosis with the determination of glycoproteins (PAG) associated pregnancy after insemination in the cows, and to determine the late embryonic mortality rates. As material, 2919 head cows in different races and ages were used in farms and family businesses in Kahramanmaraş province. Milk sample was taken on the 25-30 day after artificial insemination from cows. The level of PAG in these milk samples was analyzed by using the Milk Pregnancy Test Kit for cows. This kit was used according to the manufacturer's instructions. During the study, it was determined that 1660 cows were pregnant, 188 were suspicious and 1071 were not pregnant. The pregnant animals were examined by rectal palpation on the 50th day when their pregnancy was continued. Controls performed on the 50th day of pregnant cows showed that 1623 cows were still pregnant and 37 cows had late embryonic deaths. As a result, early pregnancies can be accurately detected by measuring the level of PAG in the cow's milk using the Milk Pregnancy Test Kit. It was concluded that late embryonic mortality rates could be detected and that this analysis could be used routinely.

**Key words:** Cow, PAG, Pregnancy, Late embryonic death

## INTRODUCTION

Pregnancy is a process starting with fertilization. The zygote, formed as a result the fertilization event, turns first into a morula with mitotic divisions, then a blastocyst (Özden, 1990; Aktumsek, 2001; Lawrence and Fowler, 2002; Senger, 2003). With the blastocyst growth, the pressure on the zona pellucida increases and rupture occurs. Then hatching takes place and the blastocyst comes out. The embryo lives as free until implantation and is fed with uterine milk (Bozdogan, 2000; Lawrence and Fowler, 2002; Hopkins, 2003; Senger, 2003).

The progesterone in the circulation allows recognition of the pregnancy on the 15th and 17th days of gestation. Another factor that provides maternal recognition is the bovine interferon-tau produced by the embryo. These two hormones prevent the secretion of PGF2α from the endometrium. Thus, pregnancy continues (Binelli *et al*., 2001; Senger, 2003). The ones described so far in the cows are in the first 3 weeks of pregnancy and this period is called early embryonic period. It is also known as the late embryonic period from 24 to 50 days of pregnancy (Santos *et al*., 2004).

Embryonic deaths are common in cattle. Embryo losses are called early embryonic death if they occur within the first 24 days of pregnancy. If pregnancy loss occurs between 24 and 50 days, it is called late embryonic death. If pregnancy loss occurs after the 50th day, it is called fetal death (Santos *et al*., 2004).

There are many factors that cause embryonic death in cows. These are genetic disorders, low quality oocyte, endometritis, hormonal environment, inadequate release of interferon tau, internal and external environmental factors, climate, stress, age, insemination time, semen quality, infectious factors and under nutrition (Gordon, 2004; Bartolome *et al*., 2006; Scott *et al*., 2011). The late rise of the level of progesterone, especially after ovulation, by effecting the development of the embryo and ability of the interferon-tau secretion leads to an increased likelihood of embryonic death (Santos *et al*., 2004).

In a study reported by Barański *et al*. (2012), embryonic mortality was found to be 9.1% between 30 and 45 days after insemination. In another study (Silke *et al*., 2002), it was determined that the embryonic mortality rate of cows in between 28th and 42nd days of gestation was 3.2% while it was 1.99% between 43th and 56th days of gestation. Humblot (2001) reported that late embryonic mortality from 6.6% to 14% in beef cattle and from 8.4% to 17.5% in dairy cattle. Another study (Diskin *et al*.,

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2011) reported that ratio of the late embryonic deaths was 10% after 28th day in Holstein-Friesian cows. Ledoux *et al*. (2015) reported that a late embryonic mortality rate was 11.7% in a study conducted on Holstein cattle.

In the above studies, late embryonic deaths were investigated and extremely different results were achieved in cows. However, in our country this issue has been investigated on very limited and few animals (Erdem, 1997; Aslan and Wesenauer, 1999). In addition, only two studies have been carried out on the determination of pregnancy-associated glycoproteins (PAGs) in the bloodstream, and no study has been carried out in our country regarding the determination of milk (Kaya *et al*., 2016; Kaya *et al*., 2017).

The aim of this study was to determine the pregnancies in cows with milk PAG tests and accordingly late embryonic mortality rates.

## MATERIALS AND METHODS

This study was conducted between June 2016 and January 2018. 2919 different cows from different races and ages were used as material between 25th and 30th days after insemination. The animals were provided from the dairy farm in Kahramanmaraş provinces and districts. The pregnancy of the cows was determined by measuring the milk PAGs level. This analysis was performed using the commercial ELISA Test Kit (Idexx Milk Pregnancy Test, IDEXX laboratories Inc, Westbrook, Maine, USA). The day of insemination was considered as day 0. Milk samples taken in the morning milk on the 25th to the 30th days of cows not showing signs of post-artificial insemination were brought to the laboratory in sterile sample containers. These milks were processed in the laboratory as described below according to the manufacturer's instructions.

All reagents were stored at 24°C before use. Into 96-well plates coated with anti-PAG antibodies; firstly 150 µl of negative control was added to 2 wells, and then 150 µl of positive control was added to the next two wells. 150 µl of milk sample was left in the remaining wells. The plate was incubated at 450 rpm, 37°C for 120 minutes by placing the shaker (BioSan Thermo-Shaker PST-60HL, BioSan, Riga, Latvia). After incubation, the plate was washed 4 times with 300 µl wash solution with washer (MW-12A, Mindray Medical International Limited, Shenzhen, China). After washing, 100 µl of detector solution was added to each well and incubated for 30 minutes. The plate was washed again 4 times. This time, 100 µl of conjugate solution was added to each well and waited for 30 minutes. At the end of the period, the plate was washed again 4 times. After washing, 100 µl of TMB substrate solution was added to the wells and incubated for 20 minutes. At the end of the incubation, the reaction was stopped by dropping 100 µl stop solution in all wells and the plate was given to the ELISA reader (Biotek ELX 800, Biotek Instrumentations, Inc, Winnooski, VT, USA). Samples and controls were measured at 450 nm and recorded. The results of milk samples were negative (OD<0.100), suspicious (0.100≤OD<0.250) and positive (OD ≥ 0.250) according to the optical densities (OD).

All animals determined to be pregnant were confirmed for pregnancy by rectal palpation 50 days after artificial insemination. Animals that were found not pregnant as a result of rectal examination were evaluated as late embryonic death.

## RESULTS

On 25-30 days after artificial insemination, 1660 pregnant, 188 suspicious and 1071 open were out of 2919 cows which were tested for pregnancy by ELISA test. Suspicious and empty cows were removed from trial. As a result of rectal examination performed on the pregnant animals on the 50th day, the pregnancy rate in the cows was determined as 97.8% (1623/1660). Late embryonic mortality was 2.2% in cows (Fig. 1, 2).

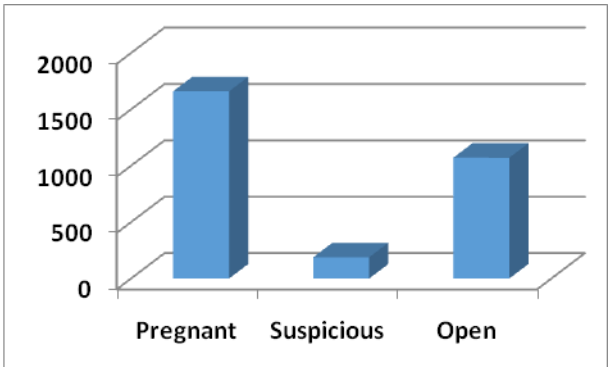


**Fig. 1:** Determination of pregnancy status in animals according to PAG level.

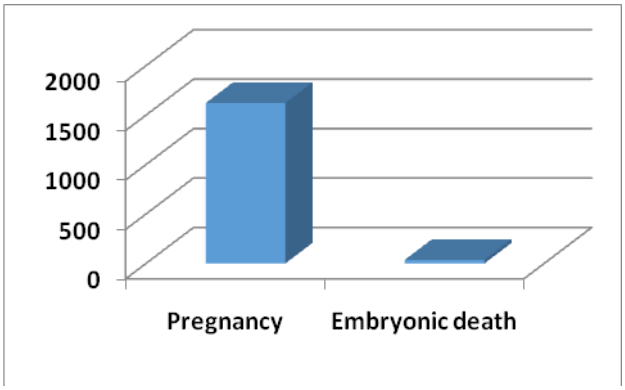


**Fig. 2:** Late embryonic deaths seen between 25-50 days in the cows.

## DISCUSSION

Maternal recognition of pregnancy in cows occurs as a result of ovarian progesterone secretion, interferon-tau production of the embryo, reduction of uterine estradiol-oxytocin receptors, and no PGF2 alpha secretion. Any problem in this mechanism results in embryonic death (Binelli *et al*., 2001). Embryonic deaths in cows are a very important reproductive health problem. In particular, embryonic deaths occurring after the 17th day of the cycle prolong the cycle return period in cows and the intervals of the estrous cycle become irregular (Gordon, 2004). For this reason, early and accurate diagnosis of pregnancy is vital for reproductive herd health. In studies investigating late embryonic mortality rates in cows, very different data are presented (Barański *et al*., 2012; Silke *et al*., 2002;

Humblot, 2001; Diskin *et al*., 2011; Ledoux *et al*., 2015; Erdem, 1997; Aslan and Wesenauer, 1999). The present study examined late embryonic mortality rates between 25-30 and 50 days of gestation.

In a study conducted by Baranski *et al*. (2012) on 954 Holstein cows in Poland, the late embryonic deaths occurring between 30 and 45 days of gestation and foetal mortality rates between 45 and 60 days have been investigated. They determined the death rates by performing ultrasound examination on the 30th day of pregnancy and rectal palpation on the 45th and 260th days of pregnancy. As a result of their study, they reported that the late embryonic death rate was 9.1% and foetal death rate was 5%. In another study conducted by Abdalla *et al*. (2017) in Egypt, late embryonic / early foetal losses were investigated in different breed cows (n=1789) and 22% offspring deaths were detected. In this present study, late embryonic mortality was detected at a lower level than both Egypt and Poland. The fact that the animals in Poland are highly productive may have caused this difference. In the Egyptian study, animals may be affected by the environment, body condition score and many other factors.

In a study conducted in Turkey (Aslan and Wesenauer, 1999), researchers investigated the early and late embryonic mortality. At the end of this study, it was reported that the early embryonic death rate was 8.8% and late embryonic death rate was 14%. In another study carried out in Turkey (Erdem, 1997), late embryonic mortality rates on 92 cows and 8 heifers were investigated. The researcher followed pregnancy in animals by ultrasonographic examination after natural or artificial insemination, and reported that the rate of late embryonic mortality was 30%. In our study, late embryonic deaths occurred at a much lower percentage than percentage found by the studies above. This may be because the number of animals used in previous studies was low.

Studies on pregnancy detection by PAG are increasingly being done (Prvanovic *et al*., 2009; Kaya *et al*., 2016; Pohler *et al*., 2016; Da Silva *et al*., 2017; Kaya *et al*., 2017; Gatea *et al*., 2018). In a study conducted on cows, Prvanovic *et al*. (2009) attempted to determine early pregnancies by measuring ultrasonography, PAGs and progesterone levels. The study concluded that all three methods were compatible with 96%. In addition, when the ultrasonic and PAG values were compared, the match was 100%. The researchers said that the pregnancy can be diagnosed by PAGs analysis on day 24. Commun *et al*. (2016) performed pregnancy detection by detecting PAG levels in serum, plasma and milk at 30, 41 and 53 days after insemination. In each sample type, they found the accuracy of the test to be over 95%. As seen in the above studies, the reliability of the test is quite high. In the present study, it was understood that all of the test-negative animals were not pregnant at the later examinations and the accuracy of the test was 100%. In addition, suspect animals were removed from trial and only cows that were confirmed to be pregnant were used.

**Conclusions**

As a result, in this study, milk amount in PAG was correctly determined in cows using Milk Pregnancy Test Kit and pregnant and non-pregnant animals were successfully detected. Today, transrectal ultrasonographic examination has to be done to detect the embryo in the early days of pregnancy. However, the use of ultrasound among veterinarians is relatively small because of the high cost of the device. Milk PAG analysis can easily be carried out by a veterinary practitioner or laboratory doing this analysis. It is advisable to use this method for the diagnosis of early pregnancies since it is low cost and easy to implement per animal. In addition, the determination of the amount of PAG in the milk and the late embryonic deaths can be clearly identified and necessary precautions can be taken to prevent these losses.

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